

Chemical composition and *in vitro* nutritional value of forage cacti (*Opuntia ficus-indica*) silage with bacterial inoculum and urea as additives

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Abstract. Possible effects of climate change, affecting rainfall patterns and longer dry spells make it necessary to research climate resilient forage alternatives for livestock feeding towards sustainable intensification, with good quality forages adapted to varied environments and hydric cycles. Forage *Opuntia* cactus may be a main component of diets for ruminants given its nutritional quality; and conserving *Opuntia* cactus as silage addresses the need to have forage year-round. Additives may enhance fermentation characteristics or the nutritional value of silage. There is a knowledge gap in the specialized literature on the effect of additives on the nutritional value of *Opuntia* silages. Therefore, the objective was to evaluate in laboratory microsilos the effect of adding a bacterial inoculant as source of homofermentative bacteria, or urea as a source of non-protein nitrogen, or the inclusion of both additives in the chemical composition and *in vitro* nutritive value of *Opuntia* silages. The treatments were: Control (TxC)= 70% forage *Opuntia* with 20% ground maize stover with ears and 10% sugarcane molasses; TxI= Control plus bacteria inoculum; TxU= Control plus 0.5% urea; and TxI/U= Control plus inoculum and urea. There were five microsilos per treatment in 4 L plastic buckets with lids that were opened after 28 days. Chemical composition of silages was determined in terms of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and *in vitro* digestibility of dry matter (IVDMD). Number and size of mold colonies, temperature and pH of silages were also determined. Treatments with urea (TxU and TxI/U) increased CP content ($P<0.05$) and IVDMD. There were no differences for number or size of mold colonies ($P>0.05$), but treatments with bacterial inoculum (TxI and TxI/U) showed lower pH ($P<0.05$). Results are valid, since experimental silos may have a more active fermentation compared to farm silos, microsilos are useful because results are like farm silos. In this experiment, results showed that *Opuntia* silages may be a forage option, and the addition of bacterial inoculum improved fermentation, and the addition of urea increased the CP content, that by also increasing IVDMD, may meet requirements for maintenance and moderate growth in cattle. It is concluded that *Opuntia* silages with 20% ground maize stover with ears and 10% of molasses (fresh basis) are a viable forage alternative for feeding ruminants, and the addition of bacterial inoculum and urea improves fermentation conditions and increases crude protein content.

Keywords: Alternative forages, forage cactus, homofermentative bacteria, non-protein nitrogen.

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Introduction

Regarding ruminant livestock production, the possible effects of climate change in central Mexico are an alteration in the rainfall patterns, with less precipitation or longer dry spells (Zamora-Martínez, 2015). This situation highlights the need for research into improved productivity in which resilient forage alternatives, better adapted to new scenarios brought about by climate change, are needed (Makkar, 2016); where forage *Opuntia ficus-indica* can be an important option given its adaptability and resistance to hydric deficit. The *Opuntia* spp. cacti originated in Mexico and Mesoamerica (Griffith, 2004). Although most of the *Opuntia* diversity is found in central Mexico (Medina-García et al., 2021), there are different species distributed in all areas of the country (CONABIO, 2023).

Forage or spineless *Opuntia ficus-indica* is used as an alternative forage in semi-arid regions in different countries; where given its resistance to dry spells and drought, has become a feeding option for ruminants (Santos et al., 2024), but not only due to its drought resistance but also its adaptability to different environments, which is greater than that of many grasses or other forage plants. In Mexico, its use has been more relevant in semidesert and desert areas (Martínez-López et al., 2009); but given its adaptability, it may be an option in other regions that currently have longer dry spells and droughts brought about by climate change, like the sub-humid temperate climate of central Mexico. Other countries like Brazil (da Silva et al., 2024), Italy (Pastorelli et al., 2022), South Africa (Magonyane et al., 2026), Zimbabwe (Gusha et al., 2015), the dry and semidry areas of the United States of America (Mayer and Cushman, 2019), and many other countries and regions (Dubeux et al., 2021) also utilize *Opuntia* as a forage source for ruminant livestock. Forage cactus provides water, energy, minerals, and vitamins; however, it must be combined with other ingredients to complete a livestock diet, as its protein content is low despite being rich in carbohydrates and calcium (Dubeux et al., 2021; Pastorelli et al., 2022).

In that sense, a favorable characteristic of ruminants is their symbiosis with ruminal microbiota, which utilize non-protein nitrogen (NPN) sources for the synthesis of microbial protein, which then passes into the rest of the ruminant host digestive tract to be used as a source of high-quality protein (CSIRO, 2007), so that sources of NNP like urea may be added to ruminant diets to increase the availability of nitrogen. Sustainable intensification of livestock production systems requires increasing productivity through appropriate feeding strategies (Makkar, 2016), based on quality forages available throughout the year. However, the availability and nutritional value of forages vary throughout the year, depending on the characteristics of each species, the agro-ecological conditions where they are produced, and their management (Martínez-Fernández et al., 2014). Therefore, it is necessary to conserve forage surpluses during peak growth periods (Wilkinson and Rinne, 2018), for their use in times of scarcity. The peak growing season for forages in the central highlands of Mexico with sub-humid climates, is the summer rainy season.

Ensiled forages are the main method of forage conservation for ruminant systems in many parts of the world (Wilkinson and Rinne, 2018). Silage is a natural fermentation process of soluble forage carbohydrates by anaerobic lactic acid-producing bacteria, which allows for the conservation of forage through acidification without negatively impacting nutritional quality (Wilkinson and Rinne, 2018; Santos et al., 2024). Additives to silages are used with the objective of enhancing the conservation or the nutritional value of the forage. There are several types of additives, fermentation boosters, fermentation inhibitors, aerobic deterioration inhibitors, or for the supply of nutrients (Mishra and Tyagi, 2024). The primary use of forage cactus has traditionally been as an emergency fodder; however, it has been proposed as an important ingredient in the diet of cattle and sheep (Pastorelli et al., 2022), and its conservation via the ensilage process has been widely considered (Santos et al., 2024).

Thus, forage cactus silage emerges as an option for providing quality fodder during periods of scarcity, or as a component of diets for ruminants. However, forage cactus has a low dry matter content, and when forages have high moisture levels and/or low levels of soluble carbohydrates, they need to be accompanied by additives to improve the fermentation conditions (Mühlbach, 2000).

In Mexico, there are few studies evaluating cactus silage, and there is a lack of scientific information on the effects of additives on the chemical composition, fermentation variables, and nutritional value of *Opuntia* silage; that calls for the evaluation of stimulant additives as lactic acid-producing (homofermentative) bacteria that are widely used to improve the quality of other forage silages (Wang *et al.*, 2019; Wang *et al.*, 2022), or the inclusion of NPN sources to improve the nitrogen availability for the synthesis of ruminal microbial protein which has been a long established practice to increase the crude protein content of diets for ruminants (Leng and Nolan, 1984).

The knowledge generated by research on the effects of additives on the nutritional value of *Opuntia* silages will provide a basis for further research on the use of this alternative forage in the feeding of livestock in different systems and areas of Mexico and abroad; which would have important implications on the quest for the sustainable intensification of ruminant livestock production by providing a source of quality forage in the dry season.

Based on the above, the research hypothesis is that the inclusion of additives may have a beneficial effect on the fermentation characteristics and nutritional value of *Opuntia* silage. The objective of this study was to evaluate, using laboratory microsilos, the effect of adding a bacterial inoculant (as a source of homofermentative bacteria to accelerate and ensure adequate fermentation during the ensiling process), a urea solution (as a source of non-protein nitrogen to increase crude protein levels), or both additives, on the chemical composition and in vitro nutritional value of forage cactus silage.

Materials and Methods

Forage sources

Cladodes of forage cactus (*Opuntia ficus-indica*) were sourced from a collaborating small-scale dairy goat farm in Yuriria, state of Guanajuato, in the Bajío region of central Mexico (20° 12' 51" N, 100° 08' 19" W). The region is a plateau with a mean altitude of 2000 m, a warm sub-humid climate with summer rains, a dry winter-spring season, and an average annual rainfall of 700 to 900 mm (INEGI, 2010). The cactus crop was planted at 1.0 m between plants and 1.5 m between rows. Fertilization was carried out with vermicompost every six months.

The cladodes were transported to the Institute of Agricultural and Rural Sciences (ICAR) of the Universidad Autonoma del Estado de Mexico (UAEM), located in the Estado de Mexico. Five laboratory microsilos per treatment (20 in total) were prepared following a methodology modified from that described by Sainz-Ramírez *et al.* (2020), using 4 L plastic buckets with lids. The cactus cladodes were chopped with a stationary forage chopper, obtaining particles with an average size of 2 cm. Each microsilo was considered an experimental unit. Although experimental silos may have a more active fermentation compared to farm silos, microsilos are useful because the results are similar to what occurs in farm silos as reported by Xiccato *et al.* (1994).

Due to the high moisture content of the forage cacti, ground maize stover with ears was added, and given the low soluble carbohydrate content, cane molasses was also added. The ground maize stover with ears was also provided by the collaborating farmer, while the sugarcane molasses was bought in. The fresh-weight proportions of the control treatment were 70% chopped cacti, 20% ground maize stover with cob, and 10% cane molasses. The weight of the forage was recorded before the silos were

made and immediately after opening the silos at 28 days, which were kept in the laboratory at an average room temperature of 21 °C.

Treatments

The evaluated treatments were Control (TxC): Control treatment without additive; bacterial inoculant (TxI): Control treatment (cactus, maize stover with ears, and molasses) plus a commercial silage bacterial inoculant composed of the homofermentative lactic bacteria *Pediococcus pentosaceus* and *Lactobacillus plantarum* at the manufacturer's recommended dose (1×10^5 CFU g⁻¹ of forage) following the recommendations for use; urea (TxU): Control treatment plus 0.5% urea (fresh weight) considering the dose used in previous trials; inoculant plus urea (TxI/U): both additives added at the doses described above; additives were dissolved in 100 mL of water and added during the preparation of the microsilos.

Chemical analysis

Samples were dried in a drying oven at 55 °C to constantly weigh for dry matter (DM), ground through a 1 mm sieve mesh. Analyses were for ash (ASH) by incineration at 550 °C (AOAC, 1990) to obtain organic matter (OM); neutral detergent fiber (NDF) with alpha-amylase addition; and acid detergent fiber (ADF) by the micro-bag method (ANKOM Technology, 2017a; 2017b), derived from the methodology described by Van Soest *et al.* (1991); ether extract (EE) (AOAC, 1990); and crude protein (CP) by the Kjeldahl method ($N \times 6.25$).

In vitro dry matter digestibility (IVDMD) was determined using the micro-bag technique with ruminal fluid in an Ankom Daisy incubator (ANKOM, 2023). Ruminal fluid was collected via nasogastric tube from three Holstein dairy cows in the final third of lactation that grazed mixed pastures for 8 h day⁻¹ between morning and afternoon milking and consumed maize silage and commercial concentrate in the evening. The rumen fluid was then pooled to form a single sample. Additionally, at the time of opening the microsilos, the number of mold colonies was counted and their diameter measured (in cm). The temperature of the ensiled forage mass was taken at three sites per microsilos using a laboratory thermometer, and pH was determined with a pH electrode.

Statistical analysis

The statistical analysis was done according to completely randomized design with four treatments and five replicates per treatment with the following model (Kaps and Lamberson, 2004) using Minitab (V14) [1].

$$Y_{ij} = \mu + T_i + e_{ij} \quad [1]$$

Where μ is the general mean, T_i is the effect due to treatments ($i = 1 \dots 4$), and e_{ij} is the residual variation. When significant treatment effects were found out ($P \leq 0.05$) the Tukey test was applied.

Results

Table 1 shows the chemical composition of the Opuntia silage treatments. Statistically significant differences ($P < 0.01$) were found for organic matter (OM), with TxU showing the highest value. Similar results were observed for crude protein (CP), which also presented significant differences ($P < 0.0001$), with higher values in the TxU and TxI/U treatments. No significant differences were found for DM,

NDF, and ADF values ($P>0.05$). The digestibility of TxU and TxI/U was significantly higher ($P<0.05$) than that of TxC and TxI, indicating that the addition of non-protein nitrogen had a positive effect. The significant differences in OM content for TxI may indicate its utilization (fermentation) by the increased bacterial population brought about by the inoculum, which is reflected in that TxI had a significant ($P<0.05$) pH (Table 2). Neutral detergent fiber (NDF) content is related to the cell wall component of forages. High fiber content may limit the digestibility and therefore, the dry matter intake. The lack of significant differences ($P>0.05$) in NDF or ADF contents indicate that the evaluated additives did not have any effect on these components.

Table 1. Chemical composition of *Opuntia* silages (*Opuntia ficus-indica*, maize stover with ears and sugarcane molasses) with additives (g kg⁻¹ DM).

Variable	TxC	TxI	TxU	TxI/U	SEM	P-Value
DM	247.06	247.11	283.78	283.20	6.78	0.18
OM	851.46 ^{ab}	843.99 ^a	861.54 ^b	859.89 ^b	2.28	**
NDF	549.82	571.30	507.38	508.54	13.1	0.228
ADF	421.73	424.02	400.02	391.27	7.43	0.334
CP	50.55 ^a	56.18 ^a	84.9 ^b	80.66 ^b	3.5	**
IVDMD	538.4 ^b	546.8 ^b	577.3 ^a	553.4 ^b	15.4	**

TxC= Control; TxI= Bacterial inoculum; TxU= Urea; TxI/U= Inoculum + urea; DM= Dry matter; OM= Organic Matter; NDF= Neutral Detergent Fiber; ADF= Acid Detergent Fiber; CP= Crude Protein; SEM= Standard Error of the Mean ^{a, b} $P<0.05$.

Table 2. Number and size (cm) of mold colonies, temperature and pH of *Opuntia* silages (*Opuntia ficus-indica*, maize stover with ears and sugarcane molasses) with additives.

Variable	TxC	TxI	TxU	TxI/U	SEM	P-Value
Mold colonies (number)	0.4	0.4	0.2	0.2	0.1	0.848
Mold colony size (cm)	4.6	6.6	0.2	4.4	1.68	0.623
Temperature (°C)	18.5	18.08	18.26	18.02	0.16	0.766
pH	4.07 ^a	3.61 ^c	4.01 ^{ab}	3.94 ^{bc}	0.05	**

TxC= Control; TxI= Bacterial inoculum; TxU= Urea; TxI/U= Inoculum + urea; SEM= Standard Error of the Mean; ^{a, b} $P<0.05$.

Table 2 shows the results for the number and size of mold colonies, temperature at opening, and pH. Mold presence was numerically higher in the TxC and TxI treatments (double the colonies on average than in the TxU and TxU/I), but differences were not statistically significant ($P>0.05$). Regarding colony size, although not statistically significant ($P>0.05$), a considerable numerical reduction was observed in the TxU treatment. Similarly, there were no statistically significant differences ($P>0.05$) in silage temperature at silo opening, which remained around the laboratory ambient temperature of 18 °C. The values for pH were around 4.0, indicating good fermentation; as a pH near 4.0 or lower favors silage conservation by limiting the growth of undesirable microorganisms (Martínez-Fernández, 2014). Highly significant differences ($P<0.001$) were observed in pH values, with the lowest values in the inoculant treatments (TxI and TxI/U), which as mentioned indicate a higher rate of fermentation from the enhanced lactic acid producing bacteria.

Discussion

Dry matter content of the silages is an important variable since high moisture contents affect the fermentation process, resulting in undesirable microorganism proliferation and a low-quality silage prone to clostridial decomposition, as well as the loss of nutrients from effluents (Martínez-Fernández, 2014).

Regarding the results for DM content, other authors in diverse countries reported higher contents (Abidi *et al.*, 2013; Miranda-Romero *et al.*, 2018; Araiza-Rosales *et al.*, 2021; Siqueira *et al.*, 2021; Bevenuto da Silva *et al.*, 2022) than those observed. Sá *et al.* (2020), in a similar experiment in Brazil, obtained lower DM values, which may have been due to the lack of an additional forage with higher DM content compared to other authors who added oat hay, olive pulp (Abidi *et al.*, 2013), or maize stover, as is the case in Miranda-Romero *et al.* (2018) and this experiment. On the other hand, Siqueira *et al.* (2021) reported very similar DM values in silage of *Opuntia* and *Nopalea cladodes* with added urea/ammonium sulphate and sugarcane bagasse. Also, the OM values obtained in the cactus silage are lower than those reported in Brazil by Siqueira *et al.* (2022) with 876 g kg⁻¹ for *Nopalea* and 887 g kg⁻¹ for *Opuntia* silage.

Higher DM content has a direct effect on silage DM intake, with higher intakes reported for dairy cows at high DM grass silages (Huhtanen *et al.*, 2007). Given the very low DM content of forage cacti, the added forages and ingredients to the silage are the main factor affecting the DM content in the *Opuntia* silages. The addition of dry forages as maize stover with ears in this experiment, or other dry ingredients (like wheat bran) are essential to obtain silages with higher DM content, reduce effluent production, and favor adequate fermentation.

The NDF contents of depends on the forage fiber content (Herrera-Torres *et al.*, 2014; Sá *et al.*, 2020; Araiza-Rosales *et al.*, 2021; Da Silva *et al.*, 2022) and lignin content. A forage with higher lignin will show higher NDF levels (Bevenuto da Silva *et al.*, 2022). However, in the experiment by Sá *et al.* (2020), NDF values were lower than those observed here, both at 60 and 120 days. Although this can be attributed to the lack of an alternative fiber source, according to the same authors, these values could have been affected over time due to hydrolysis produced by plant enzymes. As in this study, the urea treatment yielded the lowest values, which can be attributed to the alkaline hydrolysis resulting from ammonium hydroxide interacting with the ester bonds of structural carbohydrates.

Similarly, Sainz-Ramírez *et al.* (2023) in a study on faba bean silage with urea reported a decrease in fiber content. It is valid to contrast results from studies with other forages, as this is the effect that urea addition has on fiber content in any forage, although variability depends on the forage type. In another study on *Opuntia* silages, Abidi *et al.* (2013) reported NDF values like the control treatment of this study (547 g kg⁻¹ DM), as did Miranda-Romero *et al.* (2018) with 508 g kg⁻¹ DM. In a study of Ruíz *et al.* (2009) evaluating the effects of enzymes and inoculants on the chemical composition of maize silage, it was noted that treatments with bacterial inoculants obtained higher NDF and ADF values, which can be attributed to greater use of plant soluble fractions by lactic acid bacteria.

The CP results for urea-supplemented silage agree with Sá *et al.* (2020), who reported that the treatment with the highest CP value was the one supplemented with urea due to the supply of non-protein nitrogen. However, a study in Durango, Mexico, suggested that bacterial inoculants could also

increase CP values due to an increase in yeast concentration and total nitrogen content in the cactus (Herrera-Torres *et al.*, 2014). In the present experiment, treatments with urea and urea+inoculant resulted in significantly higher CP values compared to control and inoculant treatments, due to the non-protein nitrogen contribution of the urea.

Forage Opuntia has a low CP content, its main limitation as feed, which is the same situation with the maize stover with ears and the sugarcane molasses added to the silages. Therefore, the crude protein content of TxU and TxI did not meet the minimum crude protein requirements for maintenance and moderate growth of cattle (NRC, 2000) so those treatments would require protein supplementation. The addition of non-protein nitrogen as urea in TxU and TxI/U, increased the crude protein content by 55%. The intake of these silage treatments by ruminants, with increased nitrogen content, would enable the rumen microorganisms to synthesize high-quality microbial protein to meet protein requirements (CSIRO, 2007). The use of non-protein nitrogen sources like urea in this experiment to increase the crude protein contents of ruminant diets has been a long-standing practice (Leng and Nolan, 1984), and the results in this experiment meant that the CP content of TxU and TxI/U would meet the requirements for maintenance and moderate growth of beef cattle (NRC, 2000).

This increased CP content would also explain the significant increase in IVDMD in the urea treatments (TxU and TxI/U), García-Martínez *et al.* (2020) mentioned that urea-treated forages experience swelling of the fibers and rupture of lignin bonds, making them more digestible. The effect of urea addition in silage may have been greater in the ground maize stover component (García-Martínez *et al.*, 2020) and may have even affected the digestibility of the Opuntia component of the silage, since Sá *et al.* (2020) stated that because cactus has less lignin than other forages, it becomes more digestible. The increased microbial activity brought about by a higher nitrogen supply in TxU and TxI/U may also explain why IVDMD was higher in urea-supplemented treatments.

The pH levels are lower than those reported by other researchers (Abidi *et al.*, 2013; Miranda-Romero *et al.*, 2018; Sá *et al.*, 2020; Araiza-Rosales *et al.*, 2021). The values reported here are within the ideal parameter of pH around 4.0 as an indicator of good fermentation and preservation capacity (Martínez-Fernández *et al.*, 2014). The pH levels of 3.8-5.0 indicate the dominance of lactic acid bacteria and, consequently, an accumulation of lactic acid that inhibits the growth of undesirable microorganisms.

The results indicate that the inclusion of both urea as a source of NPN and the bacterial inoculum may have a complementary effect. These findings are important in the context of ruminant livestock systems in Mexico given the importance that silage from forage cacti may have both in the arid and semiarid regions, as well as in other areas that may have longer dry spells and droughts caused by the current effects of climate change. The use of the evaluated additives may also contribute to the better preservation of Opuntia silages, with the added advantage that including urea substantially increases the crude protein content of the ensiled forage.

It should be taken into consideration that the results of the present study are from an experiment undertaken in microsilos under laboratory conditions. Although evidence in the literature states that the results from laboratory microsilos are like what occurs in farm silos (Xiccato *et al.* 1994); these may require validation under commercial-scale ensiling systems.

Conclusions

Forage cactus silage supplemented with 20% ground maize stover with ears and 10% molasses exhibits good fermentative quality variables with a pH below 4.5.

Forage cactus silage is a viable fodder option for livestock systems, especially small-scale farms.

The study herein reported provides experimental evidence that shows the effects of adding an inoculum of lactic-acid bacteria, urea, or the combination of both additives on the nutritional value of the *Opuntia* silages, with a notable increase in the crude protein content of the urea containing treatments that meet the minimum maintenance and moderate growth requirements of cattle (NRC, 2000). The significant increase in the digestibility of the dry matter in the urea-supplemented treatment could also increase the dry matter intake by livestock (Huhtanen *et al.*, 2007).

The obtained results, although from a laboratory experiment, do have implications for the development of appropriate feeding strategies for ruminant livestock in the face of changing scenarios due to climate change, where *Opuntia* silages added with urea may be an option, which would require *in vivo* research that considers animal performance as well as economic and environmental appraisals.

ETHICS STATEMENT

Experimental procedures were conducted in accordance with the guidelines of the ethics and animal welfare committee of the Universidad Autonoma del Estado de México; rumen fluid was collected from three donor dairy cows using a nasogastric probe, following institutional animal welfare guidelines. No other animals or farmers were involved in the experiments (with file number DICARM-1621).

CONSENT FOR PUBLICATION

The collaborating farmer authorized the publication of results from his donation of *Opuntia cladodes* and maize stover with ears.

AVAILABILITY OF SUPPORTING DATA

Not applicable

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

A.S.-R.: Conceptualization, methodology, writing—original draft and editing. M.P.-C.: Investigation, laboratory analyses and writing. A.A.M.-C.: Investigation and laboratory analyses. F. L.-G.: Methodology, formal analyses and supervision. M.D.C.-A.: Investigation and laboratory analysis. V. H.-G.: Investigation and laboratory analyses. L.A.M.-U.: Resources and laboratory analysis. C.M.A.-J.: Conceptualization, methodology, supervision, writing – review and editing, funding acquisition.

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